endorphins from the point of view of drug development since pharmacological activity resides in the smaller fragment which is easier and more economical to synthesize. We have described the preparation and pharmacological assessment of metkephamid, an analog derived from methionine enkephalin by slight structural modification that provides enzymatic protection and bioavailability without loss of desired receptor activity. Metkephamid is more active on the mouse vas deferens preparation than methionine enkephalin, which in turn is 20 to 30 times more potent than normorphine. The observation that naloxone had the same pA_2 value versus metkephamid as it had versus methionine enkephalin is evidence that they bind to the same receptor, the δ receptor, rather than the μ receptor preferred by normorphine. Studies with guinea pig ileum and inhibition of binding in brain tissue, however, suggest that metkephamid may be more β-endorphin-like than methionine enkephalin-like (*11*); that is, it is quite active on both the μ and the δ receptors. Studies in vivo indicate that metkephamid acts on receptors in brain, which mediate analgesia, since it is more than 100 times as potent an analgesic as morphine after direct injection into the lateral ventricles. Since metkephamid is only one to three times as potent as morphine at inhibiting normorphine binding in brain (*11*) and yet is more than 100 times as potent at producing analgesia, it is likely that metkephamid utilizes a different receptor, such as the δ receptor, to mediate this activity. Indeed, metkephamid is more active than morphine on the δ receptor as indicated by the data from mouse vas deferens presented above and also the inhibition of [D-Ala^2, D-Leu^5]enkephalin (Ala, alanine; Leu, leucine) binding to brain membranes (*11*). In both δ-receptor systems, metkephamid is at least 30 times more potent than morphine.

Metkephamid is a more potent analgesic than meperidine when given intravenously, as measured by the hot plate test in mice. By the subcutaneous route, the analgesic potency of metkephamid is of the same order of magnitude as that of the standard drugs morphine, meperidine, and pentazocine, and the duration of its analgesic activity is similar to that of meperidine or pentazocine, but metkephamid produces less primary dependence than morphine, meperidine, or pentazocine. Since meperidine and pentazocine have similar analgesic activity and duration of action, the lesser dependence produced by metkephamid reflects more than mere pharmacokinetic differences. Metkephamid is also less potent than the standard drugs in suppressing withdrawal phenomena in morphine-dependent mice, rats, and monkeys and produces less respiratory depression than morphine.

The preclinical pharmacological profile of metkephamid suggests that it should be a parenteral analgesic in humans, with potential advantages over existing drugs, and it has therefore been submitted to clinical testing (*15*). The only other enkephalin analog studied in man (*21*) is FK33824, which appears to have a greater preference for the μ receptor. Preliminary data indicate that metkephamid does have analgesic activity, but it is too early to judge its efficacy and acceptability relative to other analgesics at this time.

ROBERT C. A. FREDERICKSON
EDWARD L. SMITHWICK
ROBERT SHUMAN
KERRY G. BEMIS

Lilly Research Laboratories,
Eli Lilly and Company,
Indianapolis, Indiana 46285

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Endogenous Late Positive Component of the Evoked Potential in Cats Corresponding to P300 in Humans

Abstract. *A long-latency component of the averaged evoked potential recorded from cats was present only when the evoking stimulus was relevant to the task. The amplitude of this component varied inversely with stimulus probability and was independent of stimulus modality.*

The late positive component of the human averaged evoked potential (AEP), P300, has been studied extensively since its discovery by Sutton (*1*). It is considered an endogenous component of the AEP as its appearance and amplitude depend upon the circumstances under which stimuli are presented rather than upon their physical parameters. Several investigators have shown P300 to be related to human information processing (*2*). Essentially nothing, however, is known regarding the neural processes giving rise to P300. The requisite intracranial recording and lesion experiments are very difficult to achieve using human clinical material. We report here on a long-latency evoked potential component in cats that meets experimental criteria for classification as an endogenous component analogous to P300. Possible endogenous components have been reported previously in cats and monkeys (*3*); however, these evoked potential components were not comparable to the human P300.

In humans, the P300 component (i) can be elicited by stimuli of different modalities, (ii) occurs only if the stimulus is task-relevant, and (iii) varies inversely in amplitude with stimulus probability (*4*). In addition, it is generally maintained

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that there are no substantial differences in the scalp distribution of P300 with differing stimulus modalities (*5*). These properties must also be demonstrated in experimental animals if a correspondence is to be established with long-latency components of the human AEP. We have accomplished this in cats through the use of classical conditioning to infrequent auditory and visual stimuli.

Four adult cats were prepared with an atraumatic head holder and stainless steel skull screws for recording. The recording screws were located at the midline, either 1.0 cm anterior to bregma (one cat) or 0.5 cm posterior to bregma (three cats). Surgery was carried out under sodium pentobarbital anesthesia (40 mg per kilogram of body weight).

After a recovery period of at least 1 week, subjects were trained on a classical pupillary conditioning task (*6*). They were presented with a randomized sequence of two discriminable stimuli (two tones of different frequency or a tone and a light flash). The stimuli could be varied in relative probability and in signal value as a predictor of tail shock (*7*). The stimulus having the lower probability (during habituation) or delivering information about impending tail shock was designated the "signal," the other the "background." Performance on the discrimination task was monitored by measuring pupillary dilation (*8*).

In the first task—habituation—two tone bursts of different frequency were presented at a rate of one per second. The tone of lower frequency (signal) had a relative probability of .02. No shock reinforcement was given. Habituation was considered complete when signal delivery elicited minimal or no pupillary dilation. In the second task—tone conditioning—tone bursts were delivered once every 2 seconds. The signal was followed after 700 msec by a tail-shock reinforcer. The signal probability during conditioning was .20. Conditioning required 200 to 300 signal presentations and was considered complete when pupillary dilation occurred during the interval between signal and shock on 95 percent of the trials. In the third task—light conditioning—the tone signal was replaced by a light flash. All other aspects of the task were the same as for tone conditioning.

Evoked potentials to the signal and background stimuli were amplified (gain, 2000; bandpass, 0.1 to 3000 Hz), averaged across presentations of a given stimulus, and stored on disks. During all recording sessions, the subjects were paralyzed with gallamine triethiodide and artificially respired (*9*).

After becoming conditioned to the tone, each cat was tested at least twice at each of three signal probabilities: .10, .30, and .50. Signal probabilities greater than .50 were not used to avoid presenting an excessive number of shocks. One hundred signal stimuli were presented during each test run at each probability level. Thus, for the signal, two 100-trial AEP's were obtained at each probability level. Two AEP's were obtained at each probability level for the background stimulus as well, but the number of background trials averaged varied with the probability level. Cats were then retrained to criterion using the light flash as the conditioned stimulus (light conditioning) and tested as described for tone conditioning. We did not systematically examine the effects of extinction in this experiment (*10*).

This paradigm entailed the use of aversive classical conditioning in paralyzed subjects. In contrast, human subjects in studies of P300 are verbally instructed and not restrained or aversively stimulated. Despite these differences in methods, our results were similar to those reported for human subjects. The amplitude of a positive component of the AEP occurring at 296 msec (tone signal) or 330 msec (light signal) varied inversely with stimulus probability, regardless of stimulus modality. In addition, this component was present only when the stimulus was relevant to the task, that is, when it signaled shock. In Figure 1A the response to the signal tone during habituation trials is shown along with the response to the background tone during conditioning. Both AEP's consist of a series of components in the first 150 msec, but longer-latency components are not detectable even though the tone signal was quite rare during habituation. Figures 1B and 1C show the responses to the signal stimuli (tone and light) during conditioning at each probability level, averaged over all subjects. A large positive component at about 300 msec is evident in the responses to both stimuli. Regardless of stimulus modality, this long-latency component is largest at the .10 probability level, smaller at .30 and, smallest at .50 [two-way repeated-measures analysis of variance, F (2, 6) = 46.15, $P <$ .001] (*11*). The effects of signal modality on response amplitude and the modality by probability interaction were not significant. A separate analysis of variance on the peak latencies revealed no effect of stimulus probability. The mean latency of light-evoked AEP's, however, was greater than that of tone-evoked AEP's [F (1, 3) = 14.56, $P < .05$)].

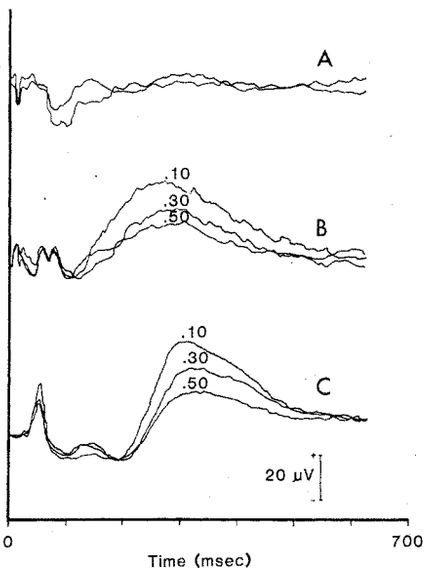

Fig. 1. Effect of stimulus probability, signal value, and modality on the amplitude of the late positive component of the AEP recorded from cats. (A) AEP's evoked by the (rare) tone signal during habituation, prior to pupillary conditioning, and by the (frequent) background tone after acquisition of the pupillary response are superimposed. (B) AEP's evoked by the tone signal after pupillary conditioning, averaged across all cats. (C) AEP's evoked by the light signal after pupillary conditioning, averaged across all subjects. AEP components in the first 200 msec differ from those evoked by the tone signal (dependent on modality). In contrast, the late positive component of the light-evoked potentials similar to that evoked by the tone signal (independent of modality).

We also examined the distribution over the skull of P300 to auditory and visual signals in three cats by recording from an array of 17 electrodes. The P300 elicited by auditory stimuli was broadly distributed, being maximal in the midline regions posterior to somatosensory areas but anterior to visual areas. The distributions were similar when visual stimuli were used, except that the largest visual P300 was slightly more posterior and lateral than that elicited by an auditory signal.

The procedures followed in this study were obviously different from those in studies of P300 in humans. Nonetheless, a late positive peak was found in the AEP's to rare, task-relevant stimuli. This peak was small or absent in the potentials elicited by rare stimuli when they were not relevant to the task. Further, its occurrence was independent of stimulus modality. Finally, its amplitude varied in a systematic fashion with changing stimulus probability. Thus, a late positive peak recorded from cats in this study behaved essentially like the P300 component recorded from humans.

The distribution of P300 across the cat's skull was not identical for visual

and auditory stimuli. However, slight differences in P300 distribution with different modalities have also been found in humans (*12*). In contrast, there are profound differences in the scalp distribution of earlier components of the AEP with changes in modality. Thus, it is likely that spatially adjacent structures contribute to the P300 component evoked by stimuli of different modalities in both the human and the cat, whereas separate neural structures are responsible for the generation of the earlier components of the AEP.

Previous research on P300 has been done exclusively on humans. However, localization of the neural generators contributing to P300 will require depth recording and lesion experiments which can be done more easily in nonhuman subjects. Likewise, investigation of neural information-processing mechanisms reflected in the P300 waveform will require detailed study of unit responses and anatomical pathways, which must be carried out in animals. This demonstration in cats of a late positive component in the AEP that behaves comparably to the human P300 provides the opportunity to obtain this important anatomical and physiological information. It is not necessarily the case that precisely the same neural processes give rise to P300 in cats and humans. However, the demonstrated similarity in the behavior of the component between species suggests that the processes might be comparable.

M. BYRON WILDER
Department of Neurology, California College of Medicine, University of California, Irvine 92717
GLENN R. FARLEY
Department of Psychobiology, University of California
ARNOLD STARR
Departments of Neurology and Psychobiology, California College of Medicine

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7. Tonal stimuli were 50-msec, 85-dB (sound pressure level) tone bursts of either 7 kHz (background stimulus) or 3 kHz (signal) delivered monaurally to the cats' left ears. Light flashes (10 msec, 32 mcd) were from a light-emitting diode (LED) placed 2.0 cm in front of the subjects' right eyes. The tail shock that followed signals during conditioning was a train of 25 current pulses (3 mA, 2 msec), delivered at intervals of 10 msec. Total train duration was 250 msec.
8. The pupillometer was similar to that used by T. D. Oleson, J. H. Ashe, and N. M. Weinberger [*J. Neurophysiol* **38**, 1114 (1975)]. It detected changes in the amount of light reflected by the iris as the cat's pupil dilated and contracted. Light was generated by three LED's and reflected by the iris to a solar cell, which generated a d-c voltage proportional to the amount of light received. This voltage was amplified with operational amplifiers and recorded on a polygraph.
9. Immobilization controlled for stimulus variables (changes in sound field and middle ear muscle contractions) and artifacts of electrophysiological origin (muscle potentials and eye movements). Gallamine acts at the motor end plate of skeletal muscle, leaving the subject conscious but unable to move. For this reason, special care was taken to minimize the discomfort of the subjects. Experimental sessions were limited to 3 hours, and cats were used no more than twice per week. Expired CO_2, cardiac rate, electroencephalogram, and body temperature were monitored to ensure that the cats were maintained in a proper state. The cats we have studied with these methods thrive. Their general condition has been satisfactory and they show no signs of fear (piloerection or hissing) when brought into the experimental chamber.
10. Other work in our laboratory has shown the P300 component to be correlated strongly with the pupil response. Both responses decrease to pretraining levels if backward conditioning (in which the shock precedes the signal) is substituted for conditioning [G. R. Farley, thesis, University of California, Irvine (1980)].
11. The AEP's recorded from the cat with the more anterior electrode placement were less than half as large as those recorded from the other three cats. For this reason we normalized the data from each cat by setting the amplitude of the late positive component elicited by the tone signal at the .10 probability level to 100 percent. The amplitude of each of the subject's other responses was then recalculated as a percentage of that response. These normalized data were used in the analyses of variance. The outcome of an analyses of variance on the raw amplitude data was similar to that obtained with the transformed data. The effect of probability was significant [$F(2, 6) = 10.04$; $P < .025$), while the effect of modality and the modality by probability interaction were not significant.
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Lunar Phasing of the Thyroxine Surge Preparatory to Seaward Migration of Salmonid Fish

Abstract. *Anadromous salmonid fish show a distinct surge in plasma thyroxine during the smoltification period prior to their migration to the sea. Analysis of 27 groups of hatchery-reared salmon and anadromous trout indicates that thyroxine levels peak coincident with the new moon. The ability to predict migratory readiness by lunar calendar would have substantial implications for the efficient culture of this economically important protein resource.*

Each year salmon and closely related anadromous trout species hatch in fresh water and, at the end of an early but variable phase of growth and development, migrate to the ocean. In most species this migration is preceded by a dramatic and essential transformation in appearance, behavior, and metabolism known as smoltification. The sum of these developmental changes results in fish which are fully suited to life in the ocean (*1*). The ability to predict when this migration will occur has long been a major concern of agencies responsible for the maintenance and enhancement of salmonid fisheries.

Knowledge of the optimal time for release of hatchery-reared fish is necessary for the efficient operation of salmon enhancement programs. Thus, many phenomena, such as scale loss, changes in coloration, body size, and gill Na^+, K^+-adenosinetriphosphatase activities, have been used as indicators of migratory readiness, but none of these has been completely satisfactory. And, therefore, the problem of when to release hatchery stock has remained, despite the intensive investigative efforts by salmonid culture agencies and research laboratories.

Smoltification may be viewed as a partial analog of amphibian metamorphosis; indeed, the hormones regulating both these phenomena together with their pattern of release may be similar. Unlike amphibian metamorphosis, however, the changes associated with smoltification are of finite duration, and, if the smolted fish are prevented from migrating to seawater, they will revert (desmoltify) to an immature parr-like condition (*1*). After reversion has taken place, young salmon that are transferred to seawater are unlikely to survive or grow. Indeed, if salmon are introduced into seawater, either prior to or following the period of smoltification, mortality is high. Of those that survive, many do not grow but appear generally weak, a condition known as "stunting" (*2*). The accurate timing of the release of hatchery-raised fish is, therefore, clearly critical to the success of these fish in seawater and ultimately to their contribution to the salmonid fishery as a whole.

Although the stunting phenomenon

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